

[0054] The present invention is not to be limited in scope by the specific examples provided for below, which are intended as single illustrations of individual aspects of the invention and functionally equivalent methods and components are within the scope of the invention.

EXAMPLE 1

Non-invasive Recovery of Sub-Stratum Corneum Cells

A. Recovery Using a Rigid Surface

[0055] Skin cells can be recovered non-invasively by scraping the skin with a sterile #15 scalpel. The scalpel is held at an angle approximately 15 degrees from horizontal and repeatedly but gently scraped across an area of skin that is approximately 1 x 1 cm in size. The epidermal cells are transferred to a sterile tissue culture well by scraping the blade against the interior wall of the well. When the glistening epidermal layer is reached, the scraping is stopped prior to causing any bleeding, to avoid contaminating the scraping(s) with blood. The cells are deposited in a sterile 1 cm petri dish and about 300 ml of lysis buffer is added to the culture well. The lysis buffer is pipetted up and down until the epidermal cells are completely lysed.

[0056] RNA lysis buffer is added within 10 minutes of initiation of the scraping. The sterile tissue culture well is maintained on dry ice. The cells are dissolved in the RNA lysis buffer, transferred into RNase free centrifuge tubes and the total RNA is extracted.

B. Recovery Using an Adhesive Surface

[0057] Skin cells can be recovered non-invasively by using Duct tape (333 Duct tape, Nashua tape products), Scotch® tape (3M Scotch® 8 10, St. Paul, MN), or D-SQUAME® (CuDerm, Dallas, TX). The skin is stripped up to a maximum 25 times. Additionally, it will be recognized that the stickier the tape, the fewer strippings are required. The skin cells were recovered by vortexing and then centrifuging the tape in an RNase-free Eppendorf tube containing lysis buffer. The same lysis buffer was reused for each piece of tape used at a single skin site. The entire procedure was performed in less than 90 minutes. The process of tape stripping itself does not affect the skin cytokine profile during the first few hours after the procedure is done.

Moreover, during the early hours after stripping no inflammatory cells migrate from the circulation into the dermis or epidermis.

[0058] RNA was immediately extracted from cells adhering to the strip by vigorously vortexing the tape in 0.5 ml TriReagent (Molecular Research Center, Inc., Cincinnati, OH). Yeast transfer RNA (4 µg) was then added as carrier RNA before the total RNA was isolated and purified according to the manufacturer's instructions. The total isolated RNA from each sample was used in an RNase protection assay (RiboQuant® Multiprobe RNase Protection Assay System, PharMingen, Inc., San Diego, CA) without prior measurement of the amount of RNA by OD measurement. Assays were performed with samples on standard acrylamide sequencing gels and used to identify digested cytokine messages. Gels containing digested RNA bands were first exposed to a Phosphor Screen (Molecular Dynamics, Inc., Sunnyvale, CA). The exposed screen was then scanned with a phosphorimager Storm 860 (Molecular Dynamics, Inc.). Intensities of bands in each sample were analyzed with the software ImageQuant™ (Molecular Dynamics, Inc.).

[0059] Appropriate care should be taken to prevent RNase contamination of the samples since skin is a rich source of RNase that can quickly degrade RNA released from damaged epidermal cells. The sample collection and extraction techniques described herein demonstrate that skin RNA can indeed be obtained without significant degradation as indicated by the ability to detect mRNA by RPA.

EXAMPLE 2

Analysis Of Cells Obtained By Tape Stripping

[0060] Irritant contact dermatitis (ICD) was induced by applying 0.5% sodium lauryl sulfate (SLS) in distilled water for 72 hours to the upper arm. After this exposure, the erythema was graded according to standard scoring sales (Fisher's Contact Dermatitis. 4th ed. Rietschel, R.L. and Fowler, J.F. Jr. eds. Williams & Wilkins, Baltimore, 1995, pg. 29). Allergic contact dermatitis (ACD) was induced by applying dibutyl squarate in acetone to the upper arm of the same subject under occlusion for 48 hours. The upper arms of the same individual (subject #1) were tape stripped 12 times and processed as described in Example 2 above.

[0061] Figure 1, lane 1 shows the RNA isolated from an ACD erythematous area of skin, read clinically as 3+ erythema, that was induced by squarate. Lane 3 is the RNA from ICD erythematous skin, clinically scored as 2+ erythema, induced after exposure to 0.5% SLS. After exposure of the x-ray film, the band for cytokine IL-4 can be clearly seen in lane 1, but not in lane 3 which contains RNA from ICD cells. Thus, the cytokine pattern in the ACD reaction clearly differed from the ICD reaction and normal skin seen in lane 2.

[0062] In a subsequent experiment, all subjects with dermatitis had mRNA encoding the cytokine IL-4 in cells from skin in areas that had demonstrated an ACD reaction (lanes 8, 11, 13 in Fig. 2). By contrast, IL-4 was not visible in any of the ICD treated areas of skin or in normal skin samples obtained from the same subjects. Furthermore, in 4 of 5 subjects (subjects 2, 3, 4 and 5 in Fig. 2), IL-8 was present in erythematous areas of skin, whether the erythema was induced by an irritant or an allergic reaction, but not in the RNA obtained from normal skin. Thus, IL-8 mRNA was generically indicative of dermatitis.

[0063] The mRNA encoding IL-13, a cytokine secreted by activated T cells, was present in 3 of the 4 erythematous areas of skin (lanes 5, 8, 11, 13 in Fig. 2) in which allergic inflammation had been induced by squarate. A faint band could be seen in the approximate area(s) expected to contain the mRNA with the molecular weight associated with gamma interferon (IFN- γ) (lanes 8 and 11 in Fig. 2). These bands were present in the mRNA extracted from 2 of the 5 squarate (ACD) treated skin samples. As was the case for IL-13, the tentative band for IFN- γ mRNA was seen in the same lanes that also had mRNA for IL-4.

[0064] IL-14, a B cell growth factor, was present in some of the squarate treated skin samples as well as some of the SLS treated skin samples (Fig. 2). IL-9, a multifunctional cytokine, was detected in all 13 samples that could be visualized in this experiment. In addition, the mRNA for the inducible isoform of nitric oxide synthase (iNOS) and IL-9 were seen in every lane that could be visualized clearly (13 of 15 samples) (Fig. 2). The presence of IL-4 in the same lanes as IL-13 strongly suggests that these two cytokine markers were induced by an allergic reaction in the skin from which the samples were obtained.

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